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REMARKS

Claims 52-75 were pending in the present application. Claims 52, 59, 66 and 71 have been amended to exclude reference to SEQ ID NO:49. Accordingly, claims 52-75 will be pending upon entry of the instant amendment. No new matter has been added, and Applicants submit that all of the claims are now in condition for allowance.

Objections to the Specification

The Examiner objected to i) the title, stating that "[i]t is not descriptive"; ii) the abstract, stating that "[i]t does not describe the claimed invention"; and iii) to the use of trademarks, such as the trademark "TAQMAN®", stating that "[i]t should be capitalized wherever it appears and be accompanied by the generic terminology."

Applicants have amended the title, the abstract and the specification to address each of the above listed objections. Therefore, Applicants believe that these objections have been obviated and respectfully request the withdrawal of these objections.

The Rejection of Claims 52-75 under 35 U.S.C. §101, Should Be Withdrawn

Claims 52-75 were rejected under 35 U.S.C §101 because the claimed invention purportedly is not supported by a credible, substantial, specific, or well-established utility. This rejection is respectfully traversed.

The standards for establishing utility sufficient to meet the requirements of 35 U.S.C. §101 are laid out in the "Utility Examination Guidelines" published in the January 5, 2001 Federal Register (hereinafter "Utility Guidelines"). 66 Fed. Reg. 1092 (2001). Specifically, the guidelines set forth two situations for satisfying the utility requirement: where Applicant has a well established utility, as it would be clear from reading the specification and claims that the invention has a well established utility, and where Applicant has asserted a specific and substantial utility that is credible. In order to make an effective rebuttal of utility, the Examiner must make a prima facie showing that either there is no well established utility or that Applicants' asserted utility is either not specific, substantial, or credible. Applicants respectfully submit that Applicants specification as filed has a well established utility. Still further, Applicants submit an asserted specific substantial and credible utility has been set forth in the

application as filed. For the reasons discussed below, reconsideration of the rejection is requested.

I. The application as filed has a well established utility.

According to the Utility Examination Guidelines:

If at any time during the examination, it becomes readily apparent that the claimed invention has a well-established utility, do not impose a rejection based on lack of utility. An invention has a well-established utility if (i) a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (e.g., properties or applications of a product or process), and (ii) the utility is specific, substantial, and credible. See MPEP §2107 II (A) (3). Contrary to the Examiner's assertions, as demonstrated below, the TANGO 294 proteins of the present invention have a well established, credible and substantial utility. Applicants respectfully submit that one of skill in the art would immediately appreciate why the invention disclosed and claimed in the present application was useful based on the characteristics of the disclosed human lipase referred to herein as TANGO 294.

Applicants point out that it is well known in the art that absorption and metabolism of fats and lipids by humans can have seriously adverse health consequences, even in the absence of an existing disease or disorder in the human. Furthermore, it is well known that lipases are one of the classes of enzymes involved in uptake, interconversion, and metabolism of lipids. It is also well established that modulation of lipase expression and activity can modulate lipid uptake and metabolism in humans, and that modulation of lipid uptake and metabolism in humans can mitigate, reduce, or prevent the adverse health consequences associated with lipid absorption and metabolism. A skilled artisan would accept that compounds identified as being capable of binding to or modulating the activity of the TANGO 294 polypeptides, could be used to modulate expression and activity of the lipase, hence possibly modulating lipid uptake and metabolism in a subject. Thus, Applicants submit that one of skill in the art would immediately appreciate the utility of the newly identified lipase as being useful in the identification of modulators of absorption and metabolism of fat and various fat- and lipid metabolism-related disorders.

II. Applicants have asserted a specific, substantial and credible utility.

The Examiner appears to question the credibility of the utilities asserted for the polypeptides of the claimed invention. An asserted utility does not meet the criterion of credibility only if it is considered to be wholly inconsistent with known scientific principles or it is speculative as to whether the attributes of the invention necessary to impart the utility are actually present in the invention. See MPEP §2107.

The Examiner suggests that Applicants have based their assertion that the TANGO 294 polypeptides of the claimed invention exhibit lipase activity by having extrapolated such utility from known lipases. The Examiner further asserts that "Almost all of the possible utilities are based on sequence homology to SEQ ID NO:49, but it is well accepted in the art that sequence homology does not impart a functional homology." Applicants respectfully disagree and assert that the lipase activity of the TANGO 294 polypeptides of the invention is not solely based on amino acid sequence homology. Additional, non-sequence-homology-based supporting evidence is provided in the specification, and Applicants respectfully contend that a skilled artisan would accept that TANGO 294 polypeptides exhibit lipase or lipase-like activity in view of all of the evidence provided in the specification.

For example, the specification discloses that, in addition to the significant overall amino acid sequence homology that TANGO 294 shares with other mammalian lipases, the amino acid sequence of the TANGO 294 protein includes specific functional amino acid sequences and residues conserved among lipases. These sequences and residues include the lipase serine active site (residues 180-189 of SEQ ID NO:47), the amino acid residues that form the catalytic triad of the lipase active site (residues 186, 357, and 386 of SEQ ID NO:47), two cysteine residues conserved among lipases (residues 260 and 269 of SEQ ID NO:47), and two conserved residues that form an oxyanion hole in lipases (residues 100 and 187 of SEQ ID NO:47).

Furthermore, Applicants have enclosed herein a phylogenetic tree that shows the relationship of the TANGO 294 amino acid sequence (identified as "Fbh46692 126 1397" and indicated with an arrow) with the top BLAST hits in a public protein sequence database (refer to Appendix A). All of the proteins shown in the phylogenetic tree for which activities have been established (indicated by stars) are lipases, sterol hydrolases (which catalyze deacylation of a sterol - a reaction analogous to lipase activity, which is deacylation of a lipid), or both. In view

of all of these similarities between TANGO 294 and known lipases (including known rat, dog and human lipases, as indicated on page 71, lines 3-9, of the specification), the skilled artisan would accept that TANGO 294 is a lipase, or at least exhibits lipase-like activity.

Also enclosed herein are a series of images of Northern blot analyses of fetal and adult human tissues (refer to Appendix B). Significant expression of TANGO 294 (designated "46691" in the Northern blots) was detected only in stomach tissue under the experimental conditions and samples used. This expression is consistent with TANGO 294 being a lipase.

III. The Examiner has not made an effective *prima facie* showing of lack of utility

In order to rebut an asserted utility, an Examiner must: *make a prima facie showing of no specific and substantial credible utility and the Examiner must establish* ***that it is more likely than not that a person skilled in the art would not consider credible any specific and substantial utility asserted*** *by the applicant for the claimed invention.* See MPEP §2107 II (C) (2). Applicants submit that the Examiner has not met the requisite requirement to rebut Applicants asserted utility. In fact, the Examiner states on page 3 of the Office Action "Based on reasonable sequence homology (50-62%) to known lipases, the protein of SEQ ID NO:47 and 49 are thought to be human lipases, which is a reasonable and acceptable asserted utility."

The Examiner states on page 3 of the Office Action that "The specification does not provide any relationship between TANGO 294 and any specific disease or syndrome or any possible use of the compounds obtained by the claimed method." Applicants assert that a showing of specific diseases which are in fact demonstrated to be treatable by the invention is not necessary. The Examiner focuses on the specific biological significance and seemingly the required efficacious use of identified compounds as Applicants requirement to satisfy the utility requirement. Applicants respectfully submit that this focus is undue and improper. Still further, the identification of targets for screening for therapeutics in the pharmaceutical industry is a well established recognized utility for enzymes useful in biological responses such as those identified and asserted in the present application.

Additionally, Applicants submit that the Examiner has not made a sufficient showing to establish, more likely than not, the utility set forth in the present specification would not be specific or substantial, as sufficient support or factual findings have not been relied upon to make

such a showing to rebut Applicants' assertion that the use in identification of therapeutics would more likely than not be useful. The Examiner makes a generic statement as to disbelief of the asserted utility and relies on general arguments to back up his claim that Applicants' original assertion is incorrect. However, this is not sufficient to meet the requisite standard that, it is more likely than not, that one of skill in the art would doubt Applicants' asserted utility. In fact, as discussed above, in view of the evidence presented in the specification and with the evidence presented herein, Applicants respectfully submit that the Examiner has not made a sufficient showing to establish, more likely than not, the utility set forth in the present specification would not be specific or substantial, as sufficient support or factual findings have not been relied upon to make such a showing to rebut Applicants' assertion that the use in identification of therapeutics would more likely than not be useful. As such, Applicants submit that the maintenance of the present rejection is improper.

Thus, Applicants submit that the application as filed sets forth that the TANGO 294 proteins of the invention have a well established, credible and substantial utility. Still further, Applicants submit that a specific, substantial and credible utility has been set forth, as described in further details above. The Examiner has not provided the preponderance of evidence required by the Utility Guidelines to establish that the utility asserted for the TANGO 294 proteins of the invention are, in view of the whole record, more likely than not, neither credible, specific, or substantial. Therefore, Applicants respectfully request reconsideration and withdrawal of the foregoing 35 U.S.C. § 101 rejection over claims 52-75.

The Rejection of Claims 52-75 under 35 U.S.C. §112, First Paragraph, Should Be Withdrawn

Claims 52-75 were rejected under 35 U.S.C §112, first paragraph, since "[t]he claimed invention is not supported by either a specific or substantial asserted utility or a well established utility", based on the rejection of these claims under 35 U.S.C §101.

For the reasons discussed above, Applicants have in fact established utility for the TANGO 294 proteins of the invention, as well as the screening assays to identify compounds which bind to or modulate the TANGO 294 proteins. Applicants therefore request

reconsideration and withdrawal of the 35 U.S.C. §112, first paragraph rejection over claims 52-75.

The Rejection of Claims 52-58 and 66-70 under 35 U.S.C. §112, First Paragraph, Should Be Withdrawn

Claims 52-58 and 66-70 are rejected under 35 U.S.C. §112, first paragraph, as "[c]ontaining subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." Specifically, the Examiner states that the "[s]pecification only provides a single representative species from human encompassed by these claims, which Applicants assert a lipase activity".

Applicants respectfully traverse this rejection. Claims 52 and 66 and dependent claims therefrom, recite polypeptides which are i) at least 90% identical to the amino acid sequence of SEQ ID NO:47; ii) at least 90% identical to residues 15-423 of the amino acid sequence of SEQ ID NO:47; iii) at least 90% identical to the amino acid sequence encoded by the cDNA insert of clone EpT294, which was deposited with ATCC as Accession Number 207220; or iv) encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 90% identical to the nucleotide sequence of SEQ ID NO:45 or SEQ ID NO:46, wherein the polypeptides disclosed in i)-iv) all exhibit lipase activity.

The limitations within these claims are fully described within the specification as Applicants have provided teachings for every element needed for one of skill in the art to practice the claimed invention. Firstly, Applicants have taught that i) polypeptide variants which retain the function of the naturally occurring protein may include sequences which are at least 90% identical to either of the two forms of the full length TANGO 294 amino acid sequence disclosed in the present application (refer to e.g. page 99, beginning at line 18); ii) polypeptide variants which retain the function of the naturally occurring protein may include sequences which are at least 90% identical to the polypeptide encoded by the cDNA insert of clone EpT294, which was deposited with ATCC as Accession Number 207220 (refer to e.g. page 3, beginning at line 7); and iii) polypeptides which are encoded by nucleic acid variants, which retain the function of the naturally occurring protein, may include sequences which are at least

90% identical to the TANGO 294 nucleic acid sequence (refer to e.g. page 93, beginning at line 1).

Secondly, as discussed above, Applicants have demonstrated that the TANGO 294 polypeptide contains several specific functional amino acid sequences and residues conserved among lipases. These sequences and residues include the lipase serine active site (residues 180-189 of SEQ ID NO:47), the amino acid residues that form the catalytic triad of the lipase active site (residues 186, 357, and 386 of SEQ ID NO:47), two cysteine residues conserved among lipases (residues 260 and 269 of SEQ ID NO:47), and two conserved residues that form an oxyanion hole in lipases (residues 100 and 187 of SEQ ID NO:47) (refer to e.g. Table X on pages 68 and 69). By having identified these regions which are necessary for activity, Applicants have taught which regions of the polypeptide are amenable to alterations as well as those which are not amenable to alterations.

Thirdly, the specification teaches one how to generate functional variants by performing conservative substitutions within the polypeptide used in the claimed invention. As defined on, for example, page 93, "[c]onservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain." The Applicants have also defined which of the amino acids have similar side chains, thereby providing a skilled artisan the necessary tools to generate functional variants of the polypeptide used in the claimed invention.

Finally, Applicants have provided teachings for one of skill in the art to be able to perform assays to determine whether or not specific sequences have the desired lipase activity. As taught on, for example, page 75 of the specification, beginning at line 15, "Lipases such as TANGO 294 catalyze formation and breakage of ester bonds between a fatty acid and a lipid moiety such as an acylglycerol, a sterol (e.g., cholesterol), and a lipoprotein. The lipases with which TANGO 294 exhibits the greatest similarity have acylglycerols and sterols among their substrates, indicating that TANGO 294 can exhibit preference for these substrates." Based on these activities, one can perform assays on specific sequences to determine whether or not such sequences have the desired biological activities. In fact, at the time of filing, numerous lipase activity assays were known in the art (see e.g., the enclosed Appendix C, comprising abstracts by

Duque et al., Hendrickson, and Bariszlovich et al.). Performing such assays to determine whether or not a variant of TANGO 294 has the desired properties would not constitute undue experimentation. Therefore, Applicants have provided all of the necessary information to enable one of skill in the art to 1) identify regions within the polypeptide used in the claimed invention which may be altered while maintaining activity; 2) generate variants; and 3) perform assays to determine whether or not the variants generated do in fact have the desired lipase activity.

Therefore, contrary to the Examiner's assertions, the specification not only provides the sequence of the polypeptide used in the claimed invention (SEQ ID NO:47), but also provides extensive teachings as discussed above, to obtain other functionally active variants which fall within the scope of the pending claims. Therefore, by having provided the full length sequence of the polypeptide used in the claimed invention and extensive teachings to permit one of skill in the art to obtain variants of the polypeptide which retain the desired function, Applicants have provided the necessary teachings to demonstrate that they were in possession of the claimed invention at the time of filing. Applicants, therefore, respectfully request reconsideration and withdrawal of the foregoing 35 U.S.C. § 112, first paragraph rejection over claims 52-58 and 66-70.

The Objection of the Specification under 35 U.S.C. §112, First Paragraph, Should Be Withdrawn

The specification has been rejected under 35 U.S.C. §112, first paragraph, "[a]s the specification lacks a sufficient written description for enablement based on deposit requirement." Specifically, the Examiner states that "[t]he applicant has deposited EpT294 under the terms of the Budapest treaty,...[b]ut there is no indication in the specification as to public availability." Applicants submit herewith a signed deposit statement, as requested by the Examiner. Therefore, Applicants respectfully request reconsideration and withdrawal of the foregoing 35 U.S.C. §112, first paragraph rejection.

The Rejection of Claims 52-75 under 35 U.S.C. §112, First Paragraph, Should Be Withdrawn

Claims 52-75 are rejected under 35 U.S.C. § 112, first paragraph, "[a]s the disclosure is not enabling for any claims." Specifically, the Examiner states that "The specification failed to identify a specific substrate by which the enzymatic activity can be assayed, or any use for the compounds that bind to or modulate the activity of TANGO 294 and its variants." The Examiner then continues by stating "While molecular biological techniques and genetic manipulation to make the TANGO294 variants are known in the art and the skill of the artisan are well developed, knowledge regarding the relationship between TANGO 294 and any disease or syndrome, an assay method for the catalytic activity of TANGO 294, the enzymatic activity of SEQ ID NO:47 or its fragment residues 15-423, or SEQ ID NO:49 is lacking."

Applicants respectfully traverse this rejection. The Examiner states that it would require undue experimentation on the part of one of skill in the art to practice the claimed invention. To demonstrate this point, the Examiner describes each step that one would be required to perform to practice the claimed invention. The first step described by the Examiner is "[t]he search for a TANGO 294 having 90% sequence identity to SEQ ID NO:47 or 49." As discussed at length above, this step would not constitute undue experimentation because the specification fully describes how one would go about identifying TANGO 294 variants which retain lipase activity. The second step described by the Examiner is "[i]dentify its biological or chemical activity and its association with a disease or syndrome". Again, as discussed above, the variants generated would only be selected if they demonstrate the desired lipase activity. This activity could easily be assessed by performing specific lipase assays which were known in the art at the time of filing (see e.g., the enclosed Appendix C, comprising abstracts by Duque et al., Hendrickson, and Bariszlovich et al.). As for the association of TANGO 294 polypeptides with a disease or syndrome, as discussed above, Applicants assert that a showing of specific diseases which are in fact demonstrated to be treatable by the invention is not necessary. The Examiner focuses on the specific biological significance and seemingly the required efficacious use of identified compounds. Applicants respectfully submit this focus is undue and improper. The third step described by the Examiner is "[d]evelop an assay method for the catalytic activity". As discussed earlier, the activity of the TANGO 294 or TANGO 294 variant would necessarily be a

lipase activity. In fact, this is a limitation of the claims. As for developing an assay to assess lipase activity, many such assays were already known in the art at the time of filing, hence the skilled artisan would not need to develop such assays, but simply perform known assays. Finally, the last step described by the Examiner is "[i]dentify compounds that bind or modulate the catalytic activity and identify a disease or syndrome which said compounds can treat". The specification fully enables one of skill in the art to perform screening assays to identify compounds capable of binding to or modulating the activity of the TANGO 294 polypeptides (see e.g. section beginning on page 129 of specification). Again, Applicants respectfully submit that the Examiner's requirement that the identified compounds be associated with a specific disease or syndrome is undue and improper.

Therefore, contrary to the Examiner's assertions, Applicants have provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of pending claims 52-75. Therefore, Applicants respectfully request reconsideration and withdrawal of the foregoing 35 U.S.C. § 112, first paragraph rejection over claims 52-75.

CONCLUSIONS

In view of the amendments and remarks made herein, Applicants respectfully submit that the objections and rejections presented by the Examiner are now overcome and that this application is now in condition for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

It is believed that this paper is being filed timely and that a two month extension of time is required. In the event any additional extensions of time are necessary, the undersigned hereby authorizes the requisite fees to be charged to Deposit Account No. 501668.

Entry of the remarks made herein is respectfully requested.

Respectfully submitted,

July 19, 2004

MILLENNIUM PHARMACEUTICALS, INC.

By _____

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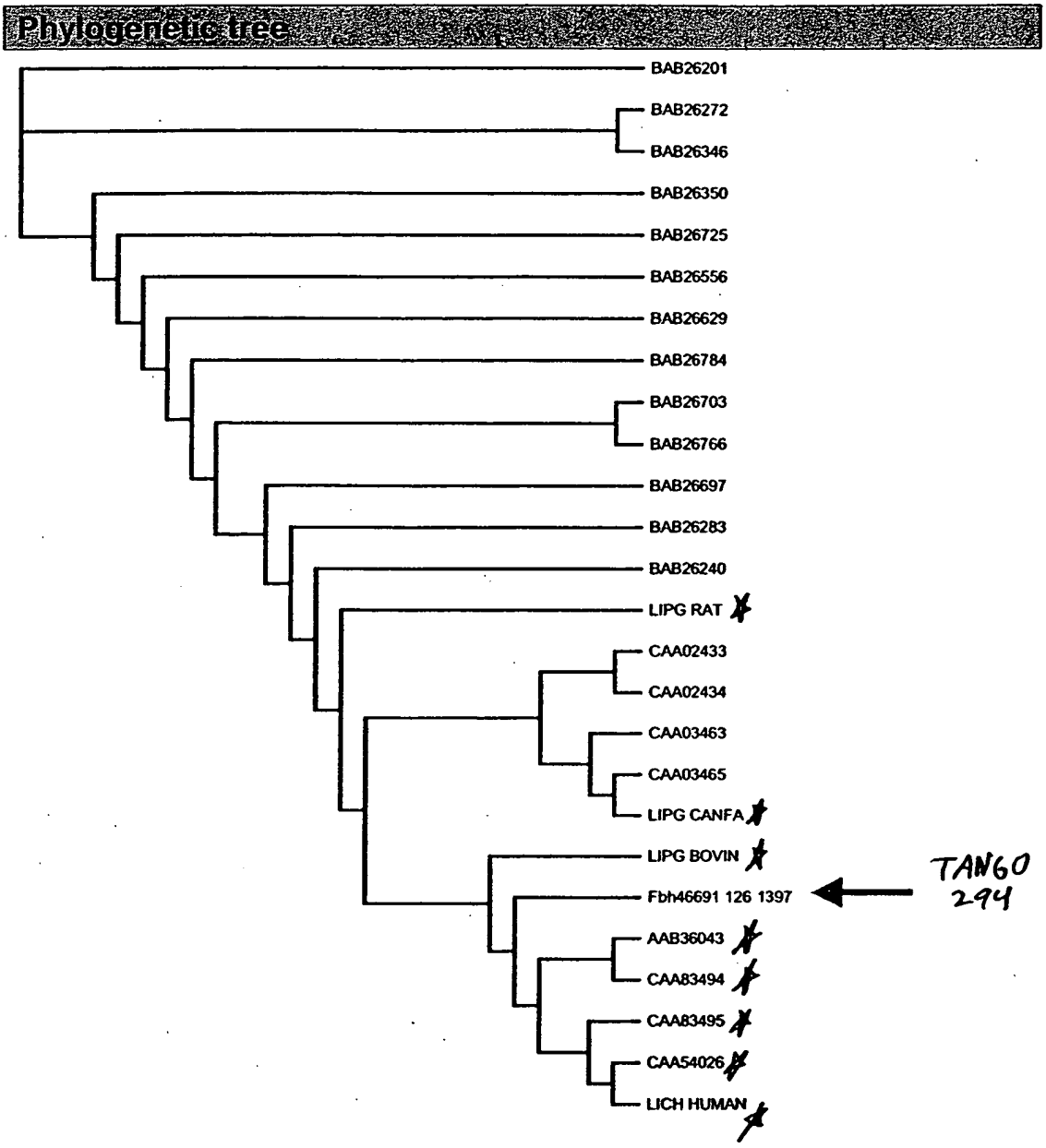

see Key on next page for identities of proteins.

Full BLAST Report

Program: BLASTP 2.0MP-WashU [07-Jun-2001] [sol2.6-ultra-ILP32F64 17:41:00 07-Jun-2001]
Query= Fbh46691_126_1397 - Import - vector trimmed
(423 letters)
Database: protot 692,327 sequences; 220,281,154 total letters.
References:

- Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. J. Mol. Biol. 215: 403-10.
- Altschul et al. (1997), Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. 25: 3389-3402.
- **Program Descriptions:** BLAST2 | WU-BLAST2 | Help Manual

HTML formatting provided by the Bioperl Blast module.

Sequences producing High-scoring Segment Pairs:	High Score	Probability P(N)	N
emb|CAA54026| (X76488) sterol esterase [Homo sapiens] gb...	1290	2.8e-131	1
emb|CAA83495| (Z31690) lysosomal acid lipase [Homo sapien...	1290	2.8e-131	1
sp|P38571|LICH_HUMAN LYSOSOMAL ACID LIPASE/CHOLESTERYL ES...	1289	3.5e-131	1
gb|AAB36043| (S81497) lysosomal acid lipase; LAL [Rattus ...	1220	7.3e-124	1
emb|CAA83494| (Z31689) lysosomal acid lipase [Mus musculus]	1196	2.5e-121	1
sp|P04634|LIPG_RAT TRIACYLGLYCEROL LIPASE, LINGUAL PRECUR...	1166	3.8e-118	1
sp|P80035|LIPG_CANFA TRIACYLGLYCEROL LIPASE, GASTRIC PREC...	1154	7.2e-117	1
emb|CAA03463| (A57756) unnamed protein product [unidentif...	1144	8.2e-116	1
emb|CAA03465| (A57760) unnamed protein product [unidentif...	1144	8.2e-116	1
sp|Q29458|LIPG_BOVIN TRIACYLGLYCEROL LIPASE, PREGASTRIC P...	1142	1.3e-115	1
emb|CAA02433| (A39301) unnamed protein product [unidentif...	1135	7.4e-115	1
emb|CAA02434| (A39303) unnamed protein product [unidentif...	1135	7.4e-115	1
dbj|BAB26283| (AK009431) putative [Mus musculus]	1134	9.4e-115	1
dbj|BAB26556| (AK009875) putative [Mus musculus]	1134	9.4e-115	1
dbj|BAB26697| (AK010093) putative [Mus musculus]	1134	9.4e-115	1
dbj|BAB26725| (AK010139) putative [Mus musculus]	1134	9.4e-115	1
dbj|BAB26201| (AK009300) putative [Mus musculus] dbj|BAB...	1133	1.2e-114	1
dbj|BAB26272| (AK009413) putative [Mus musculus] dbj|BAB...	1133	1.2e-114	1
dbj|BAB26766| (AK010203) putative [Mus musculus]	1131	2.0e-114	1
dbj|BAB26629| (AK009990) putative [Mus musculus]	1129	3.2e-114	1
dbj|BAB26346| (AK009537) putative [Mus musculus]	1128	4.1e-114	1
dbj|BAB26240| (AK009359) putative [Mus musculus]	1127	5.2e-114	1
dbj|BAB26350| (AK009544) putative [Mus musculus]	1126	6.6e-114	1
dbj|BAB26784| (AK010231) putative [Mus musculus]	1126	6.6e-114	1
dbj|BAB26703| (AK010103) putative [Mus musculus]	1125	8.5e-114	1
dbj|BAB26359| (AK009560) putative [Mus musculus]	1124	1.1e-113	1
dbj|BAB26651| (AK010026) putative [Mus musculus]	1124	1.1e-113	1
dbj|BAB26287| (AK009437) putative [Mus musculus]	1122	1.8e-113	1
sp|P07098|LIPG_HUMAN TRIACYLGLYCEROL LIPASE, GASTRIC PREC...	1119	3.7e-113	1
dbj|BAB26733| (AK010148) putative [Mus musculus]	1119	3.7e-113	1
dbj|BAB26704| (AK010106) putative [Mus musculus]	1116	7.6e-113	1
dbj|BAB26746| (AK010173) putative [Mus musculus]	1115	9.7e-113	1
emb|CAA29414| (X05997) gastric lipase precursor [Homo sap...	1111	2.6e-112	1
emb|CAA03464| (A57758) unnamed protein product [unidentif...	1006	3.4e-101	1
emb|CAA94824| (Z70780) contains similarity to Pfam domain...	757	8.4e-75	1
gb|AAK68537|AC024835_3| (AC024835) Hypothetical protein Y5...	754	1.7e-74	1
gb|AAG45574| (AF067942) similar to lysosomal acid lipases...	753	2.2e-74	1
emb|CAB01973| (Z79696) predicted using Genefinder-Similar...	727	1.3e-71	1
dbj|BAB31766| (AK019504) putative [Mus musculus]	715	2.4e-70	1
gb|AAC69088| (AF022976) Hypothetical protein R11G11.14 [C...	706	2.1e-69	1
gb|AAC48051| (U64849) Contains similarity to Pfam domain:...	693	5.1e-68	1
emb|CAC27244| (AX068259) unnamed protein product [Homo sa...	685	3.6e-67	1
sp|O46108|LIP3_DROME LIPASE 3 PRECURSOR (EC 3.1.1.-). em...	663	7.7e-65	1
emb|CAB02896| (Z81055) Similarity to Human triacylglycero...	653	8.8e-64	1
gb|AAF52971| (AE003629) CG6113 gene product [Drosophila m...	649	2.3e-63	1
gb|AAF52985| (AE003629) CG17099 gene product [Drosophila ...	639	2.7e-62	1
gb|AAF58163| (AE003811) CG8093 gene product [Drosophila m...	629	3.1e-61	1
gb|AAC62229| (AF063014) yolk polypeptide 2 [Plodia interp...	623	1.3e-60	1
gb|AAF56528| (AE003754) CG5990 gene product [Drosophila m...	576	2.2e-55	1
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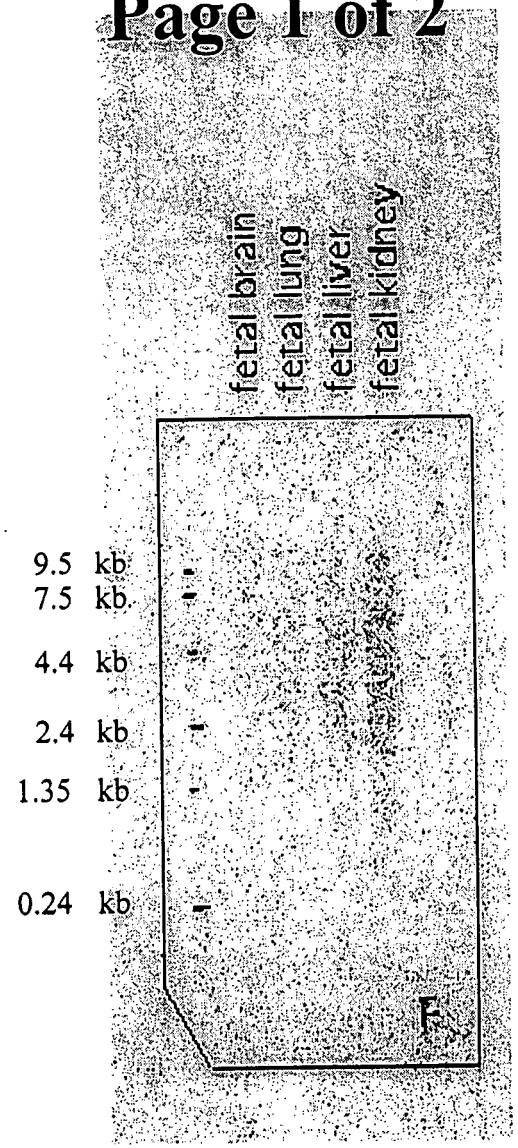
46691 Expression in Fetal and Adult Tissues

Page 1 of 2



46691 Expression in Fetal and Adult Tissues
Page 2 of 2

1: J Lipid Res 1996 Apr;37(4):868-76

New fluorogenic triacylglycerol analogs as substrates for the determination and chiral discrimination of lipase activities.

Duque M, Graupner M, Stutz H, Wicher I, Zechner R, Paltauf F, Hermetter A.

Department of Biochemistry and Food Chemistry, SFB-Biokatalyse, Technische Universitat Graz, Austria.

A new type of fluorogenic and isomerically pure 1(3)-O-alkyl-2,3 (3,2)-diacyl glycerols was synthesized that can be used as substrate for the determination of lipase activities. These compounds contain a fluorescent pyrene acyl chain and, as a potent quencher of pyrene fluorescence, a trinitrophenylamino acyl residue. In their intact form, the fluorogens show only low fluorescence intensity. Upon lipase-induced or chemical hydrolysis of the substrates, however, the fluorophore and quencher separate from each other. This leads to a gradual increase in pyrene fluorescence, reflecting the time-dependent progress of lipolysis and, under substrate saturation conditions, lipase activity. This lipase assay is continuous and does not require separation of substrate and reaction products. Short- and long-chain homologues as well as optical isomers of the fluorogenic alkyldiacyl glycerols were hydrolyzed by pancreatic lipase, hepatic lipase, and lipo-protein lipase at highly different rates depending on the substrate or enzyme preparation and source (e.g., postheparin plasma or cultured cells). It is proposed that a useful set of enantiomeric and/or homologous substrates in combination with appropriate reaction media might be applied to the selective determination of a lipase in a mixture of lipases, e.g., hepatic and lipoprotein lipase in PHP, for medical diagnostics.

PMID: 8732786 [PubMed - indexed for MEDLINE]

1: Anal Biochem 1994 May 15;219(1):1-8

Fluorescence-based assays of lipases, phospholipases, and other lipolytic enzymes.

Hendrickson HS.

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In choosing an assay, one needs to consider the following questions: What level of sensitivity is required? Must the assay be continuous? Is the substrate readily available; can it be purchased or must it be custom synthesized? How specific is the substrate? How convenient is the method? How compatible is it with monomolecular, micellar, or vesicular substrates? How tolerant is it of added detergents and proteins that may be present? What is the cost of substrates, fluorescent probes, and instrumentation? Of the many methods described in this review, discontinuous assays using natural substrates and derivatization of the products with fluorescent probes are probably the most reliable and most tolerant of reaction conditions. A drawback is the involvement of tedious and time-consuming steps which limit the number of trials that can be performed. Continuous assays, in which changes in fluorescent properties of the probe are monitored, are most convenient for kinetic studies, although they are also most sensitive to reaction conditions and intolerant of added detergents and proteins. One has to carefully consider all of these issues and choose a method best suited to the enzyme, the particular information one wants to obtain, and the availability of substrates, probes, and instrumentation. Hopefully, increased commercial availability of fluorescent substrates and probes will make these choices easier. Nevertheless, the search goes on for better, more sensitive and convenient fluorescent assays.

Publication Types:
- Review
- Review, Tutorial

PMID: 8059934 [PubMed - indexed for MEDLINE]

1: Nahrung 1990;34(8):701-17

Related Articles, Books, LinkOut

[The characterization of microbial lipases. 1. The determination of lipase activity]

[Article in German]

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In the selection of an appropriate method for activity determination of lipases existing technical equipment, kind of enzymes, number of samples investigated (e.g. in routine analysis), and expected sensitivity range have to be taken into account. Titrimetric methods and above all copper salt methods with their high detection sensitivity are the most suitable procedures for activity determination of lipases used in laboratories and institutions without equipment for radiochemical analysis.

Publication Types:
- Review
- Review, Academic

PMID: 2233988 [PubMed - indexed for MEDLINE]
